PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 27 July 2000 (27.07.00)	
International application No. PCT/EP99/09989	**Applicant's or agent's file reference** 0480/001203
International filing date (day/month/year) 16 December 1999 (16.12.99)	**Priority date** (day/month/year) 16 December 1998 (16.12.98)
Applicant GARCIA-LADONA, Francisco, Javier et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

13 June 2000 (13.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Facsimile No.

Form PCT/IB/331 (July 1992)

EP0000020

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

GOLDSCHEID, Bettina
Basf Aktiengesellschaft
67056 Ludwigshafen
ALLEMAGNE

Date of mailing (day/month/year) 24 October 2000 (24.10.00)	
Applicant's or agent's file reference 0480/001203	**IMPORTANT NOTIFICATION**
International application No. PCT/EP99/09989	**International filing date** (day/month/year) 16 December 1999 (16.12.99)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
GARCIA-LADONA, Francisco, Javier Raiffeisenstrasse 9 D-76870 Kandel Germany	ES	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
GARCIA-LADONA, Francisco, Javier Brehmstrasse 107 i 76870 Kandel Germany	ES	DE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

[ ] [illegible]
[X] the International Preliminary Examining Authority

[X] [illegible]
[ ] other

[illegible]
34, chemin des Colombettes
1211 Geneva 20, Switzerland

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below	
0480/001203		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/09989	16/12/1999	16/12/1998

Applicant

KNOLL AKTIENGESELLSCHAFT et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readble form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2 ☒ **Certain claims were found unsearchable** (See Box I)

3 ☒ **Unity of invention is lacking** (see Box II)

4 With regard to the **title,**

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5 The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant

☒ None of the figures

INTERNATIONAL SEARCH REPORT

national application No.
PCT/EP 99/09989

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-2 relate to an extremely large number of possible methods. In fact, the claims include so many possible compounds that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely the mechanism of action per se (modification of homer expression) and the use of the suggested compounds for treating the defined medical indications .

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1 -2

   Use of a homer expression modifying compound for the treatment of a neuroleptic syndrome or psychosis, especially schizophrenia

2. Claim : 3

   Use of a homer expression modifying compound for the treatment of an oncological disorder

3. Claims: 4-7

   Use of a compound which interacts with the metabotropic receptors in the treatment of different disorders such as psychosis and neuroleptic induced disorders

4. Claim : 8

   Certain nucleic acids

5. Claims: 9-10

   Screening methods for finding compounds modifying homer expression using certain cells

6. Claim : 11

   Use of a homer expression modifying compound for the treatment of CNS disorders

7. Claims: 12-29

   Use of a homer expression inducing compound or a homer peptide interacting with the homer interaction motif located in the disease-associated-target for the treatment of different diseases

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/09989

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/00 A61K31/4465 C12N15/63 C07K14/47 G01N33/53
A61P25/18 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 40407 A (WORLEY PAUL F ;BRAKEMAN PAUL R (US); UNIV JOHNS HOPKINS (US)) 17 September 1998 (1998-09-17) page 18, line 17-19 page 2, line 34 page 3, line 2 -page 4, line 2 ---	1-3
X	JAMES E F REYNOLDS: "MARTINDALE THE EXTRA PHARMACOPOEIA" 1996 , ROYAL PHARMACEUTICAL SOCIETY , LONDON, GB XP002135213 page 714-715: "Haloperidol" -----	1-3

☐ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the

10 April 2000

13. 06 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/09989

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9840407 A	17-09-1998	AU 6702698 A	29-09-1998
		EP 0970119 A	12-01-2000

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
0480/001203		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/09989	16/12/1999	16/12/1998
International Patent Classification (IPC) or national classification and IPC A61K31/00		
Applicant KNOLL AKTIENGESELLSCHAFT		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

13 06 2000

Name and mailing address of the international	Authorized officer

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/09989

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-20 as originally filed

Claims, No.:

1-29 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-29.

because:

☒ the said international application, or the said claims Nos. 1-2 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 3-29.

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Inventive step (IS) No Claims 1-2

Industrial applicability (IA) Yes Claims

No: Claims 1-2

2. Citations and explanations
see separate sheet

To section III

Claims 1-2 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

To section V

1. Reference is made to the following documents:
   D1: WO 98 40407 A (WORLEY PAUL F ;BRAKEMAN PAUL R (US); UNIV JOHNS HOPKINS (US)) 17 September 1998 (1998-09-17)
   D2: JAMES E F REYNOLDS: 'MARTINDALE THE EXTRA PHARMACOPOEIA' 1996 , ROYAL PHARMACEUTICAL SOCIETY , LONDON, GB

2. The subject-matter of the present claims 1 to 2 cannot be considered as novel since it is already known to use haloperidol, which is a Homer expression modifying compound, in the treatment of schizophrenia. It is pointed out that finding the mode of action cannot make the use novel, neither can it be considered as an invention. The requirements of Article 33 (2) have therefore not been fulfilled.

3. D1 discloses that compounds which modify the Homer expression can be used in certain brain disorders. For the skilled person it is obvious to use said compounds also in the treatment of neuroleptic disorders and psychosis. The subject-matter of the present claims 1 to 2 therefore also lacks the necessary inventive step (Art 33 (3) PCT).

4. For the assessment of the present claims 1-2 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claim. The EPC [illegible] claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 31/00, 31/4465, C12N 15/63, C07K 14/47, G01N 33/53, A61P 25/18, 35/00

A3

(11) International Publication Number: **WO 00/35430**

(43) International Publication Date: 22 June 2000 (22.06.00)

(21) International Application Number: PCT/EP99/09989

(22) International Filing Date: 16 December 1999 (16.12.99)

(30) Priority Data:
98123943.7 16 December 1998 (16.12.98) EP

(71) Applicant *(for all designated States except US)*: KNOLL AKTIENGESELLSCHAFT [DE/DE]; D-67061 Ludwigshafen (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GARCIA-LADONA, Francisco, Javier [ES/DE]; Raiffeisenstrasse 9, D-76870 Kandel (DE). LANG, Sandra [DE/DE]; Bgm-Grünzweig-Strasse 24, D-67063 Ludwigshafen (DE).

(74) Agent: GOLDSCHEID, Bettina; Basf Aktiengesellschaft, D-67056 Ludwigshafen (DE).

(81) Designated States: CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(88) Date of publication of the international search report:
14 September 2000 (14.09.00)

(54) Title: HOMER A NEW TARGET OF TREATING PSYCHIATRIC DISORDERS

(57) Abstract

A method for treatment of psychosis, schizophrenia, oncological disorders, tumors and/or CNS disorders in a human being comprising administering to said human being a composition comprising an effective amount of a compound which interacts with homer or metabotropic receptors are disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
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BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	[illegible]
[illegible]		[illegible]		[illegible]		[illegible]	
CN	[illegible]	**KR**	[illegible]	**PT**	[illegible]		
CU	[illegible]	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	[illegible]		
DE	[illegible]	[illegible]		[illegible]			

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/09989

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/00 A61K31/4465 C12N15/63 C07K14/47 G01N33/53
A61P25/18 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	WO 98 40407 A (WORLEY PAUL F ;BRAKEMAN PAUL R (US); UNIV JOHNS HOPKINS (US)) 17 September 1998 (1998-09-17) page 18, line 17-19 page 2, line 34 page 3, line 2 -page 4, line 2 ---	1-3
X	JAMES E F REYNOLDS: "MARTINDALE THE EXTRA PHARMACOPOEIA" 1996 , ROYAL PHARMACEUTICAL SOCIETY , LONDON, GB XP002135213 page 714-715: "Haloperidol" -----	1-3

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex

° Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu-

10 April 2000

13.06.00

Name and mailing address of the ISA

Authorized officer

INTERNATIONAL SEARCH REPORT

International application No
PCT EP 99/09969

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1 ☐ Claims Nos.
because they relate to subject matter not required to be searched by this Authority, namely:

2 ☒ Claims Nos. –
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3 ☐ Claims Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1 ☐ As all required additional search fees were timely paid by the applicant, this international Search Report covers all searchable claims.

2 ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3 ☐ As only some of the required additional search fees were timely paid by the applicant, this international Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4 ☒ No required additional search fees were timely paid by the applicant. Consequently, this international Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

FURTHER INFORMATION CONTINUED FROM PCT/ISA 210

1. Claims: 1 -2

Use of a homer expression modifying compound for the treatment of a neuroleptic syndrome or psychosis, especially schizophrenia

2. Claim : 3

Use of a homer expression modifying compound for the treatment of an oncological disorder

3. Claims: 4-7

Use of a compound which interacts with the metabotropic receptors in the treatment of different disorders such as psychosis and neuroleptic induced disorders

4. Claim : 8

Certain nucleic acids

5. Claims: 9-10

Screening methods for finding compounds modifying homer expression using certain cells

6. Claim : 11

Use of a homer expression modifying compound for the treatment of CNS disorders

7. Claims: 12-29

Use of a homer expression inducing compound or a homer peptide interacting with the homer interaction motif located in the disease-associated-target for the treatment of different diseases

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-2 relate to an extremely large number of possible methods. In fact, the claims include so many possible compounds that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear (and/or concise), namely the mechanism of action per se (modification of homer expression) and the use of the suggested compounds for treating the defined medical indications .

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Inter...al Application No
PCT/EP 99/09989

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9840407 A	17-09-1998	AU 6702698 A	29-09-1998
		EP 0970119 A	12-01-2000

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 31/00, 31/4465, C12N 15/63, C07K 14/47, G01N 33/53, A61P 25/18, 35/00

A2

(11) International Publication Number: **WO 00/35430**

(43) International Publication Date: 22 June 2000 (22.06.00)

(21) International Application Number: PCT/EP99/09989

(22) International Filing Date: 16 December 1999 (16.12.99)

(30) Priority Data:
98123943.7 16 December 1998 (16.12.98) EP

(71) Applicant (*for all designated States except US*): KNOLL AKTIENGESELLSCHAFT [DE/DE]; D-67061 Ludwigshafen (DE).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): GARCIA-LADONA, Francisco, Javier [ES/DE]; Raiffeisenstrasse 9, D-76870 Kandel (DE). LANG, Sandra [DE/DE]; Bgm-Grünzweig-Strasse 24, D-67063 Ludwigshafen (DE).

(74) Agent: GOLDSCHEID, Bettina; Basf Aktiengesellschaft, D-67056 Ludwigshafen (DE).

(81) Designated States: CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: HOMER A NEW TARGET OF TREATING PSYCHIATRIC DISORDERS

(57) Abstract

A method for treatment of psychosis, schizophrenia, oncological disorders, tumors and/or CNS disorders in a human being comprising administering to said human being a composition comprising an effective amount of a compound which interacts with homer or metabotropic receptors are disclosed.

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Homer a new target of treating psychiatric disorders

Schizophrenia is a chronic psychiatric disorder affecting approximately 1% of the adult population. The economic and social cost of schizophrenia and other psychotic disorders are considerable due to the large index of hospitalization. The real causes of schizophrenia remains still unknown. The symptoms, classified as positive (hallucinations) and negative (social withdrawal, paranoia) may be observed in some cases as early as in adolescence. Schizophrenic patients suffer a progressive degradation of mood, though and cognition processes (Wright I. and Woodruff P., 1995). Compounds with a beneficial effect on the treatment of schizophrenia or psychosis have been so-called neuroleptics. Early studies suggested that an alteration of the brain dopaminergic system may be related to schizophrenic and psychotic symptoms. Although the alteration of dopaminergic function in the brain of schizophrenics is evident, whether the onset of the disease is due to this alteration or it is only a delayed consequence of the disorder remains unknown. An intense research has been developed regarding the brain dopaminergic system and the pharmacology of dopamine receptors. Typical antipsychotics, such haloperidol, with a strong therapeutic effect on the treatment of psychosis have high affinity to D2 dopamine receptors (Seeman 1987). However, this property is associated to a high incidence of extrapyramidal side effects in most of the cases in a irreversible form (Gratz S.S. and Simpson G.M. 1994; Ebadi M. and Srinivasan S.K. 1995). In addition, haloperidol have also high affinity for sigma receptors supporting them as a therapeutic target for the treatment of psychosis (Reynolds G.P. and Czudek C. 1995). Drugs specific for other brain receptors have been also proposed as antipsychotics (Fatemi H. et al., 1996). Atypical antipsychotics with mixed pharmacological profile, like clozapine, has been very useful for an effective and safer treatment of psychosis. However, to date, no fully efficient treatment have been found for the treatment of neither psychosis nor neuroleptic malignant syndrome.

It has been demonstrated that dopamine receptor blockade after acute treatment with neuroleptics induces genomic responses in

D2-receptors. The genomic response induced by neuroleptics may be involved not only to their beneficial effects of antipsychotics

The knowledge of gene expression changes induced by neuroleptics may help to understand both the beneficial and side effects of antipsychotic drugs and therefore also to define new and more effective targets for the treatment of schizophrenia and psychosis. The aim of the invention was to disclose genomic effects induced by neurolpetics and subsequently to identify new targets for the treatment of psychotic and neurodegenerative disorders.

Recently it has been shown that gene expression of a synaptic protein (homer) can be up-regulated by different stimulus such treatment with the neurostimulant cocaine, seizures or brain synaptic activity particularly during the development (Brakeman P.R. et al., 1997). Homer protein is new 28 kd synaptic protein which coding gene has been sequenced (Ozawa K.A. et al., 1997; Brakeman P.R. et al., 1997). The amino acid sequence contains a PDZ-domain. Homer protein shares only a 10% homology with other members of the PDZ-family thus establishing a putative new group. Homer protein is able to interact with the intracytoplasmic part of metabotropic glutamate receptor proteins mGluR1A and mGluR5 (Brakeman P.R. et al., 1997). These excitatory aminoacid receptors are coupled to excitotoxic mechanisms in brain (Knöpfel T. and Gasparini F. 1996). The precise role of homer in the central nervous system is not yet elucidated. However, the fact that homer protein contains a PDZ domain strongly suggests, as for other proteins containing such domain, the possibility of interaction with other cellular proteins involved in cell signaling systems (Pointing C.P. and Phillips C., 1995) and not only to metabotropic receptors.

The present invention provides the identification of cell systems useful for the study of homer function and as tools for the discovery of new therapeutic compounds related to this protein. The present invention provides human homer gene sequences.

The present invention provides the evidence that homer gene expression can be up regulated in vivo by the treatment with haloperidol.

The present invention also provides the partial sequence of

The present invention is also directed to the effect of antipsychotic treatment on homer gene expression and the identification

psychotic disorders in general. The present invention also concerns the identification of homer protein, metabotropic and sigma receptors as targets for the identification and preparation of medicinal compounds useful for the treatment of neurodegenerative processes such as senile dementia of Alzheimer's type, argirophilic grain disease and other senile dementias in general, Parkinson's disease and atypical forms of Parkinson's disease, Huntington's disease, demyelinating diseases such multiple sclerosis, progressive multifocal leukoencephalopaty, infection-induced demyelinization, and demyelinization disorders of genetic origin, amyotrophic lateral sclerosis and HIV induced dementia. The present invention also provides a new target for the treatment of CNS diseases with a evident glial cell reaction. The present invention provides new therapeutic targets for the treatment of leukemia and brain tumors.

Regulation of homer gene expression by haloperidol

Methods

Animals

Adult Sprague-Dawley rats (250 g) were maintained in normal environmental conditions with free access to food and water ab libitum.

Treatment

Animals were treated with haloperidol (0.5 and 5 mg/kg) or saline. Naive (non-treated) animals were used as additional controls. Animals were also treated with amphetamine or with amphetamine and MPEP or SIB-1893 as described in example 18b.

Example 1. Tissue preparation.

The animals were sacrificed by decapitation 90 minutes after treatment. Whole brain was rapidly removed from the skull, frozen in dry ice and stored at -30°C. Rat brain sections (15 µm) were obtained at -20°C in a cryostat, mounted in gelatine-coated slides and stored at -30°C until used.

[illegible] synthesis and labelling of oligonucleotides

[illegible] accession number [illegible], [illegible] was homerA1 [illegible]-CTCGAGTGCTGAAGATAGGTTGTTCCCCCATTTTC-CCCA-[illegible] antisense oligonucleotide

was complementary to bases 894-934. The corresponding sense oligonucleotides (homerAS and homerBS) were used as control probes. Synthesis was performed in a 395 Applied Biosystems DNA Synthesizer. Purified oligonucleotides were dissolved in DEPC-treated water and stored at -30°C until use. Labelling of synthetic oligoprobes was performed using a deoxynucleotidyl-transferase (TdT) labelling kit (NEP-100, NEN, Bad-Homburg, Germany). Briefly, 5 pmol of probe were incubated (2 hr) with TdT (36 units) in presence of 50 pmol of [^{35}S] dATP (NEN) and Cl_2Co. The reaction was stopped and the labelled oligonucleotides purified by column chromatography. Labelling efficacy was checked by paper chromatography in phosphate buffer system.

Example 3. In situ hybridization histochemistry

Homer gene expression in rat brain was studied using in situ hybridization techniques well known in the art. In situ hybridization was performed as previously described (Garcia-Ladona et al., 1994). Briefly, Tissue sections were fixed with paraformaldehyde in PBS and treated (min) with pronase (0.25 mg/l), rapidly washed, dehydrated by consecutive incubation with 60%, 80%, 90% and 100% ethanol, rapidly dried and used for hybridization. Brain sections were incubated with 100 µl of 10 mM Tris-HCl hybridization buffer (pH: 7.5) containing 50% formamide, 0.6 M NaCl, 1x Denhardt's solution, 1 mM EDTA, 0.58 mg/ml yeast t-RNA, 10% dextran sulphate, 10 mM DTT and [^{35}S]-labelled oligoprobe (13500 c.p.m./µl). A nescofilmR strip was deposed over hybridization mixture to avoid evaporation and the slides incubated overnight at 42°C in a humid chamber. Afterwards, hybridization solution was washed out from the slides and non specific hybridization was eliminated by incubation (4x 1 hr) with 10 mM Tris-HCl ph:7.5 containing 0.6 M NaCl and 1 mM EDTA at 60°C. Nucleic acids in the sections were precipitated by two consecutive washes with 70% and 95% ethanol containing 0.3 M ammonium acetate. The slides were dried and exposed with a X-O-mat X-ray film (Kodak). The films were developed after 48 hr. Measurements of autoradiographic images were performed with an image analysis system equipped with SigmaScanpro software (Jandel Scientific).

[illegible] penicillin [illegible] unit/ml and streptomycin [illegible] mg/ml

Culture conditions were 95% humidity and 5% CO_2. Cell population was splited 1 3 every 3 days.

The culture of glioma A-172 cells (ATCC) was performed using conditions commonly used by the art. Cells were grown in medium containing special supplement (DMEM NUT F-12) containing serum (10%), penicilin and streptomycin. Culture conditions were 37°C, 95% humidity and 5% CO_2. Cells were grown till confluence and then splited.

The culture of glioma U87 cells (ATCC) was performed using conditions commonly used by the art. Cells were grown in medium containing special supplement (DEMEM NUT F-12) containing serum (10%), penicilin and streptomycin. Culture conditions were 37°C, 95% humidity and 5% CO_2. Cells were grown till confluence and then splited.

Example 6. Culture of glial cells

The culture of glial cells was performed by methods clearly described in the art. Whole brains from new born rats were dissected out of the skull in aseptic conditions. Brain areas (striatum, cortex and hippocampus) were dissected and immersed in culture medium without serum. Small pieces of tissue were obtained by scretching with micro forceps. Tissue was homogenated by 10 passages through a 1.2 mm gauge. Cell suspensions were centrifugated and the pellet resuspended in culture medium and plated in pedri dishes. Cell were incubated for 2-3 hr at 37°C, 95% humidity, 5% CO_2. Non-attached cells were aspirated and centrifugated. Pelleted cells were resuspended in medium containing 10% serum and antibiotics and then plated in petri dishes for 10-20 days. Medium was renewed every 3-4 days.

Example 7. RNA isolation

The isolation of RNA from different cell and tissue sources was performed using a single-solution extraction method commercially available (Trizol®, Gibco Life Sciences). The cells were washed with RNAse free PBS and the homogenized with Trizol®. The total [illegible] was determined by measuring light

Homer gene expression in human glioma cells A-172, U87, in [illegible] was studied

of common use in the art. A commercially available RT-PCR kit was used (Ready to go, Amersham/Pharmacia Biotec). Protocols were adjusted to the supplier recommendations. RT-PCR was performed using 1 mg total RNA. Primers were selected by analysing the homer gene sequence (accession number: U92079) logged in the GENEBANK. Thermocyler was programmed as follows (RT 30 min at 42°C, PCR 23 cycles of 1 min 95°C, 1 min 60°C, 2 min 72°C). At the end of PCR cycles, samples of PCR mixture were electrophoresed in agarose gels as described by commonly used protocols.

Example 9. Analysis of nucleic acids by gel electrophoresis

Gels for the analysis of RT-PCR products were prepared by melting agorose (1%) in electrophoresis buffer (Current Protocols in Molecular Biology, John Wiley & Sons, 1995) at 60°C. PCR samples were mixed with sample buffer containing and loaded (1µg/lane). Electrophoresis was run 60 min. and separation of fragments was checked by u.v illumination.

Example 10. Analysis of gene sequences

The analysis of gene sequences obtained by RT-PCR was performed by using software commercially available or in the public domain. The sequence identification was performed by homology search using DNASIS software (HITACHI) and software available in the public GENEBANK.

Results

Homer gene expression is up regulated after antipsychotic treatment.

The in situ hybridization images showed that homer mRNA transcripts were present in higher levels in haloperidol treated animals than in controls (Fig. 1) The differences, on optical density measured in autoradiographic films, between control and treated animals are shown in figure 2. Homer gene expression induced by treatment with amphetamine is reduced in brain frontal cortex by administering compounds MPEP and STB-1893.

and the [illegible] sequences fully agree in their size with the expected values [illegible] of RT-PCR products

gene. Homology analysis demonstrate some punctual differences with the rat homer gene sequences (see apendix 1).

Homer gene is expressed by Chinese Hamster Ovary Cells.

The fragments of DNA obtained after RT-PCR using RNA from CHO cells and specific primers complementary to homer gene sequences fully agree in their size with the expected values of the homer gene fragments. The sequencing of RT-PCR products demonstrate their identity as sequences located in the homer gene. Homology analysis demonstrate some punctual differences with the rat homer gene sequences (see appendix 1).

Homer gene is expressed by human eritroleukemic (HEL) cells.

The fragments of DNA obtained after RT-PCR using RNA from HEL cells and specific primers complementary to homer gene sequences fully agree in their size with the expected values of the homer gene fragments. The sequencing of RT-PCR products demonstrate their identity as sequences located in the homer gene. Homology analysis demonstrate some punctual differences with the rat homer gene sequences (see appendix 1)

Example 11 provides a method to detect the efficacy of antisense oligonucleotides in cultured cells.

Cells were cultured as described in example 4. Cells were treated for different times with different concentrations of antisense, sense and missense oligonucleotides complementary to human homer gene sequences. The presence of homer protein were determined by western blots (example 13) and immunocytochemistry (example 12) using specific antibodies directed against human homer polypeptide sequences. The effects of antisense oligonucleotides complementary to human homer gene sequences were determined by using different methods of to determine second messenger signal pathways activation (see examples 14, 15 and 16)

Example 12 provides a method to detect homer protein by immunocytochemistry. The method was similar to that previously [illegible] (Garcia-Sadona 1997)

previously [illegible]

Example 14 provides a method to determine the agonist-induced phospholipase C activity.

The method was basically described previously (Garcia-Ladona et al., 1993). Cells were incubated for 24 hr with 0.125 µM [^{3}H]myoinositol. Non incorporated [^{3}H]myo-inositol was eliminated from the medium and replaced with Krebs-Henseleit buffer containing 10 mM LiCl. After 10 min incubation, different agonist were added for 45 min. The reaction was stopped by replacing the stimulation medium with distilled water. In the case of tissue samples, the procedure is very similar (Garcia-Ladona et al., 1993) Cells were frozen and stored at -80°C. Production of [^{3}H]myo-inositol monophosphate was determined in cell samples by known methods of chromatographic purification (in Methods in Neutrotransmission receptor analysis. Eds H.I. Yamamura, S.J. Enna M.J. Kuhar, Raven Press, 1990). A similar method to determine agonist-mediated phospholipase C stimulation was used by preparing membrane fractions and incubating with [^{32}P]PIP_2 and agonist or antagonists. In this case the production of IP3 was determined. Methods known of the art have been also optimized for using microtiterplates based systems. Commercially available materials allows to perform high throughput and secondary screening (Amersham pharmacia biotech and NEN).

Example 15 provides a method to determine agonist-induced elevation of intracellular Ca^{++} levels.

The method used was similar to known methods described in the literature (Nuccitelli R, 1994). Briefly cells were grown in culture bottles as indicated (example 4). Cells were softly scraped before reaching confluence. Cell were labelled with Fura-2 by incubating (30 min) with Fura-2-acetyl-methylester at room temperature. Cells were centrifuged at 180 x g for 10 min and resuspended in DMEM-F 12 medium without serum and incubated at 37°C, 5% CO_2 and 95% humidity for 45 min. Intracellular calcium levels were determined in a fluorescence microscope equipped with an appropriate filter exchange system (Olympus / Hamamtsu). Fluorescence ratio (A340 / A380) was measured using Argus^R software. Intracellular calcium levels were monitored in single cells for a short period in the absence of drugs and then for

Example 16 provides a method to measure agonist-induced cAMP production in cultured cells.

The method was similar to that used currently in the art (In Methods in Neurotransmission receptor analysis. Eds H.I. Yamamura, S.J. Enna, M.J. Kuhar, Raven Press, 1990). Briefly, cells were incubated for 10 min in culture medium in the absence of serum and antibiotics. Reaction was stopped by heating to 95°C 15 min. Cell samples were frozen and stored at -80°C. cAMP levels were determined with commercial available kits (Biotrak from Amersham) using the cAMP binding protein. Methods known of the art have been also optimized for using microtiterplates based systems. Commercially available materials allows to perform high throuphput and secondary screening (Amersham and NEN).

Example 17 provides a method to determine the effects of antisense oligonucleotides in vivo.

Antisense nucleotides were dissolved in physiological saline and injected intravenously and intracerebroventricularly in animals. Different periods of treatment were established. After treatment, animals were sacrificed and different organs and body fluids used for histological analysis. Animals were used to determine the effects on the homer and other cellular proteins production by using immunohistochemical, immunocytochemical and western blot methods described in the examples 12 and 13 respectively. Animals were also used to determine the effect on cell signalling processes by methods exemplified in the examples 14 and 16. A group of animals were tested in behavioural models for antipsychotic effects (see examples).

Example 18a provides a method to determine the efficacy of a compound in an animal model for prediction of antipsychotic activity.

The method have been reported in the literature by injecting PCP in animals. Animals were treated before and after PCP with different doses of compound. Afterwards a set of animals were used in behavioural models predictive of psychotic activity to asses compound's efficacy (example 28). An additional set of

Example 18b:

Methamphetamine antagonism was tested by recording methamphetamine-induced hyperactivity (measurement of locomotor activity).
Mice (NMRI, 21-26 g; female) received drug or vehicle, intraperitoneally, 30 min prior to methamphetamine (MET, 1 mg/kg po). Locomotor activity was recorded in cages equipped with light beams (2 mice/cage/dose) for 1 h, starting 30 min after MET. For calculation of drug effects the counts recorded during the time period of 15 to 60 min after start of the measurement were selected. The control value was calculated as the difference between the counts recorded for the methamphetamine group and the vehicle-treated group during the same time period.

Cataleptogenic effects
The cataleptic syndrome was tested according to the method described by Wirth et al. (Arch. Int. Pharmacodyn. Ther. 115, 1-31, 1958). The animals (male rats, Sprague-Dawley bodyweight 210-225 g; n/dose=4) were regarded as cataleptic if they remained in an abnormal posture for more than 15 sec, i.e. one foreleg an a 9-cm-high piece of cork. The animals were tested 30m 60, 120, 180 and 300 min after intraperitoneal administration of the test compound.

Results
Table

Compound	Methampnetamine antagonism ED50 [mg/kg ip]	Cataleptogenic effect [x/n] at dose [mg/kg ip]
BSF 470213	53.4	0/4 at dose 100
BSF 470214	51.2	0/4 at dose 100

The test compounds showed a dose-dependent antagonism of methamphetamine-induced hyperactivity. No induction of catalepsy was found.

The compounds used are SIB 1893 (2-methyl-6-(2-phenylethenyl)-

Example 19 provides a method to determine the efficacy of compounds on preventing neuroleptic induced malignant syndrome.

Different animals models are may be used including haloperidol induced catalepsy and chronic treatment with haloperidol. Animals may be subsequently used to determine behavioural deficiencies (example 28, anatomical neurodegeneration and changes in gene expression (as exemplified in examples 1-3, 12, 13).

Example 20 provides a method to determine the efficacy of a compound in the treatment of demyelinating diseases. Different animals models of demyelinization are known of the art. Demyelinization was induced by injecting antibodies. Animals were treated with the compounds after the induction of myelin loss. Animal brains were used to determine the levels and integrity of myelin.

Example 21 provides a method to determine the efficacy of a compound in the treatment of demyelinizating diseases.

The method consist in the use of oligodendrocytes-enriched cell cultures from normal and demyelinizated animals (jimpy mutation) as described (Garcia-Ladona et al., 1997). Cells were treated with different doses of the compound and the integrity of myelin sheets and the levels of myelin markers were determined.

Example 22 provides a method to predict efficacy of a compound in Parkinson's disease. Different models were used, MTP induced Parkinsonism in mice, 6-OHDA induced degeneration in substantia nigra (Drug Discovery and Evaluation, Eds. H.G. Vogel and W.H. Vogel 1997).

Example 23 provides a method to determine the beneficial effects of a compound in senile dementia of Alzheimer type.

The method is known of the art and consists on the use of transgenic animals overproducing b-amyloid protein. Animals can be treated with compounds and analysed for memory deficits and other behavioural parameters.

Example 24 provides a method to [illegible]

Binding saturation kinetics of a radioligand. Membranes (200 µl) were incubated (600 µl total volume) in 100 mM Tris-HCl (pH: 7.7) containing 1 mM EDTA (buffer B) with increasing concentrations of radioligand in the presence (non specific binding) or in the absence (total binding) of an antagonist at high concentration. Incubation was prolonged for 90 min at 30°C; afterwards, samples were filtered, using a Skatron filtration system, through GF/B filters embedded in 0.3% poly-ethylenimide. Filters were washed with 9 ml of butter B at 4°C. Radioactivity retained in the filters was measured by liquid scintillation counting using 5 ml Ultima-Gold.

Displacement of radioligand binding: Binding displacement experiments were performed basically as reported in other studies. Membranes (200 µl) were incubated in buffer B (600 µl total volume) with increasing concentrations of the selected compounds in the presence of a selected concentration of radioligand. After a 87 min incubation period at 30°C, samples were filtered with buffer B at 4°C through GF/B filters. Filters were washed with 9 ml buffer B. Radioactivity retained in the filters was determined as above. Total binding was defined as radioligand binding observed in the absence of other compounds. Non specific binding was defined as radioligand binding levels observed in the presence of antagonist in high concentration.

Analysis of radioligand binding data Saturation parameters radioligand were estimated both by no-linear regression analysis and from linear plots by using SigmaPLot software (Jandel Scientifivc Germany). Displacement curves were build from radioactive binding levels expressed as percentage of total binding. IC_{50} and Hill coefficients (n_H) were estimated by non linear regression analysis.

Example 15 provides a method to identify compounds with agonist activity at different receptors by measuring agonist stimulated $[^{35}S]GTP\gamma S$ binding.

The methods are very well known in the art (Hilf and Jakobs 1992). Briefly, agonist activity was determined by measuring drug-induced changes of $[^{35}S]GTP\gamma S$ binding in membranes from cells. Cell membranes were obtained

[illegible] mM NaCl, 1 mM DTT, 1 mM EDTA, 10 µM GDP and $[^{35}S]GTP\gamma S$ (nM). Following 60 min incubation, at 30°C, in the absence or in the presence of [illegible]

(100 µl) was rapidly filtrated through GF/B filters using a Skatron® filtration device. Filters were quickly washed with 9 ml of 50 mM Tris-HCl buffer (4°C) containing 100 mM NaCl, 5 mM $MgCl_2$ at pH: 7.5. Radioactivity retained in the filters was determined by scintillation spectrometry using Ultima-Gold scintillation liquid. Drug activities were expressed as % of basal binding levels measured in the absence of the compound. Curves were fitted using a non-linear regression analysis software (Sigma Plot, Jandel Scientific, GErmany) to the general equation $E = (L*E_{max}) (L+EC_{50})$ where E is the effect, L is ligand concentration, E_{max} is the maximal effect and EC_{50} is the concentration inducing 50% of the maximal effect.

Example 26 provides a method to prepare cell membranes.

Different methods have been described in the art (Biologiacal Membranes, Eds). Membranes were prepared form cell cultures. Cells were softly scraped from bottle surface and centrifuged 10 min at 180 x g. Cell pellets were resuspended in 5 mM Tris HCl buffer (pH: 7.6), containing 5 mM EDTA, 5 mM EGTA, 0.1 mM PMSF and 3 mM benzamidine (buffer A) and incubated for 15 min at 4°C. Cell suspension was homogenized (6 x 3s) in an Ultraturrax (15000 r.p.m.) and centrifuged for 1 min at 1000 x g and 4°C. Nuclear pellet was resuspended in buffer A, homogenized and centrifuged as above. Supernatants of both steps were collected and centrifuged for 20 min at 40000 x g at 4°C; pellet was resuspended in buffer A and homogenized (1 x 15s). Membrane suspension was centrifuged for 20 min at 40000 x g at 4°C. The resulting pellet was resuspended in buffer A containing 10% glycerol and 1% bovine serum albumin. Aliquots were frozen and stored at -80°C until use.

Example 28 provides a method to study psychosis by using animal models.

The method has been described in the literature (Swerdlow N.R. et al., 1996). Animals may be treated by different routes with compounds and checked for behavioural parameters to determine efficacy as antipsychotic agents (Drug Discovery and Evaluation, Eds H.G. Vogel and W.H. Vogel, 1997).

The method is very well known in the art (Lipska M. et al., [illegible] Animals are lesioned with a excitotoxin in the ventral

adulthood. Animals may be treated with compounds and checked for different behavioural parameters including prepulse inhibition paradigm (example 28).

The term human homer gene refers to polynucleotide sequences with homology to the so-called rat homer gene. The term human homer protein refers to a peptide resulting of the translation of human homer gene sequences using the natural code.

The present invention provides four partial nucleotide sequences of human homer gene.

The present invention also provides partial nucleotide sequences of homer gene of hamster and homer protein expressed by astrocytes in culture.

Nucleic acid sequences according with the present invention can be used to design anti-sense oligonucleotides and to determine aminoacid sequences of polypeptides encoded by them.

Modified antisense oligonucleotide synthesis is well known in the art (Gene Therapy, Eds, J.T. August, 1997). Different oligonucleotide modifying groups can be used.
Modified anti sense oligonucleotides will be tested to determine time-life, bioavailability an efficacy on inhibiting the homer protein translation (examples 11, 12 and 13).

The human homer peptides of present invention can be used to raise specific antibodies. The present invention also includes the use of antibodies or antisense oligonucleotides raised against human homer protein as therapeutic compounds and as probes to detect human homer protein and gene respectively (see examples 1, 3, 12 and 13). Where probes means unlabelled or isotope or non-isotopically labelled compounds that bind to a specific target. The antibodies against the human homer sequence can be obtained using the known protein chemistry techniques.

The present invention also includes a method to disclose the role of homer protein in neurotumoral and leukemic cells for example in invasive activity, proliferation, cell survival, apoptosis.

[illegible] homer protein on the activity of other cell signalling mechanisms by using HEL cells and A-172 and [illegible] human glioma cells

The present invention includes a method to study the role of human homer protein on the activity of specific cell proteins and receptors by transfecting their genes in HEL cells, A-172 and U87 human glioma cells and CHO cells. Tranfection techniques are well known of persons skilled in the art and involve the transference into the cell of gene sequences included in a vector. Where vector means polynucleotide sequences that facilitate the insertion in a host of a given genetic information. Where vectors include plasmids and eucaryotic viruses and bacteriophages. Many vectors and expression systems are well known and documented in the art (for example pcDNA3, pCR2.1 from Invitrogene).

Methods to detect human homer

The present invention includes a method to detect human homer protein in human brain by using antisense oligonucleotides or antibodies indicated above. Examples of such methods are reported in examples 1, 2, 3, 12 and 13).

The present invention provides a method to detect human brain (glial) tumors by using antibodies directed against human sequences of homer or using isotope- or non-isotopically-labelled oligonucleotide probes complementary to human homer sequence. Methods are exemplified in examples 1, 2, 3, 12 and 13.

The present invention includes a method to detect human glioblastome and leukemic cells in culture using antibodies directed to human homer protein or antisense nucleotides complementary to human homer protein gene sequences. Methods for such detection are reported in examples 2, 3, 4, 12 and 13.

Neurodegenration and homer

The present invention includes a method to treat human brain degenerative processes by using compounds modifying homer gene expression. Methods to identity such compounds are exemplified in the examples.

Where degenerative processes are ischemia of vascular origin, ischemic states induced by brain or spinal cord trauma, epilepsy, psychotic disorders [illegible]

[illegible] brain cells. Degenerative processes is a term used also as synonym of neurodegeneration and or neurodegenerative disease.

The present invention also provides a method for treating or prevent neurodegeneration by using compounds facilitating (modifiying) the interaction between homer and other components of the cell signalling pathways, genetic information or cellular proteins. Where genetic information is any DNA or RNA sequences present in the cell.

The present invention also provides a method for treating or prevent neurodegeneration by using compounds facilitating the interaction between homer and metabotropic receptors. Such compounds may be identified by coincubating membranes from cells expressing metabotropic receptors, purified human homer protein, antibodies directed to homer protein and by using a commercial methods (SPA, Amersham or flashplates) to detect binding activities.

The present invention also provides a method for treating or prevent neurological deficits observed in patients suffering of neurodegenerative diseases by using agonist/antagonist of metabotropic receptors. Such compounds may be identified using current membrane binding methods as described.

The present invention also provides a method for treating or prevent neurological deficits observed in patients suffering of schizophrenia or any other psychotic disorders by using antagonists of metabotropic receptors. Such compounds will be identified using membrane binding methods described in the examples. Suitable compounds are those used in example 18b.

The present invention also contains a method to treat and prevent neurological deficits induced after treatment with typical antipsychotics by using antagonist/agonist of metabotropic receptors. Such compounds will be identified using membrane binding methods described in the examples.

The present invention contains a method to modify the expression of homer protein by using compounds with affinity to sigma or dopaminergic receptors. The compounds will be selected by using in the examples. Their efficacy will be determined by using the methods described in examples 1, 2, 3, 6, 7, 8 and 9.

The present invention provides a method to treat human [illegible] (glial) tumors by using modified or unmodified antisense [illegible] complementary to human homer mRNA sequences

or by using compounds modifying the expression of homer protein acting directly in the transcription or in the translation, protein folding, protein maturation, protein turnover processes, or by using compounds that modify the interaction between homer and any other cellular protein or peptide included those involved in signalling processes, and genetic information. Where genetic information is DNA or RNA sequences. Compounds may active by different treatments including intravenous application, orally treatment or stereotaxically injected in the brain tumor area.

The present invention provides a method to treat leukemias by using modified or unmodified antisense oligonucleotides complementary to human homer mRNA sequences or by using antibodies directed against human homer protein, or by using compounds modifying the expression of homer protein acting directly in the transcription or in the translation, protein folding, protein maturation, protein turnover processes, or by using compounds that modify the interaction between homer and any other cellular protein or peptide included those involved in signalling processes, and genetic information. Where genetic information is DNA or RNA sequences. Compounds may active by different treatments including intravenous application and oral treatment.

Another subject of the invention is a method for treatment of specific diseases by administering to a human being in need thereof a composition which comprises an effective amount of a compound which induces the homer protein expression. The so induced homer expression products interact with targets which are associated with the respective disease. Another embodiment for the interaction with the disease-associated-target is to administer polypeptides comprising a sequence form the homer expression product. These polypeptides (homer peptides) interact with the homer interaction motif of the respective disease-associated target. The diseases and the corresponding disease-associated targets are disclosed in the claims.

Appendix 1
Sequence of homer gene amplified from HEL mRNA and its correspondign aminoacid sequence

A CTCGAGCTCA TGTCTTCCAA ATTGACCCAA ACACAAAGAA GAACTGGGTA
CCCACCAGCA AGCATGCAGT TACTGTGTCT TATTTCTATG ACAGCACAAG AAATGTGTAT
AGGATAATCA GTTTAGATGG CTCAAAGGCA ATAATAAATA GTACCATCAC CCCAAACATG
ACATTTACTA AAACATCTCA GAAGTTTGGC CAGTGGGCTG ATAGCCGGGC AAACACCGTT
TATGGATTGG GATTCTCCTC TGAGCATCAT CTTTCGAAAT TTGCAGAAAA GTTTCAGGAA
TTTAAAGAAG CTGCTCGACT AGCAAAGGAA AAATCACAAG AGAAGATGGA ACTTACCAGT
ACACCTTCAC AGGAATCCGC AGGCGGGGAT CTTCAGTCTC CTTTAACACC GAAAGTA

STRAHVFQID PNTKKNWVPT SKHAVTVSYF YDSTRNVYRI ISLDGSKAII NSTITPNMTF
TKTSQKFGQW ADSRANTVYG LGFSSEHHLS KFAEKFQEFK EAARLAKEKS QEKMELTSTP
SQESAGGDLQ SPLTPKVXG

Appendix 2 Homer gene sequence amplified from U87 mRNA and its corresponding amincacid sequence

A TGGGGGAGCA ACCTATCTTC AGCACTCGAG CTCATGTCTT CCAAATTGAC
CCAAACACAA AGAAGAACTG GGTACCCACC AGCAAGCATG CAGTTACTGT GTCTTATTTC
TATGACAGCA CAAGAAATGT GTATAGGATA ATCAGTTTAG ATGGCTCAAA GGCAATAATA
AATAGTACCA TCACCCCAAA CATGACATTT ACTAAAACAT CTCAGAAGTT TGGCCAGTGG
GCTGATAGCC GGGCAAACAC CGTTTATGGA TTGGGATTCT CCTCTGAGCA TCATCTTTCG
AAATTTGCAG AAAAGTTTCA GGAATTTAAA GAAGCTGCTC GACTAGCAAA GGAAAAATCA
CAAGAGAAGA TGGAACTTAC CAGTACACCT TCACAGGAAT CCGCAGGCGG GGATCTTCAG
TCTCCTTTAA CACCAGAAAG TA

MGEQPIFSTR AHVFQIDPNT KKNWVPTSKH AVTVSYFYDS TRNVYRIISL DGSKAIINST
ITPNMTFTKT SQKFGQWADS RANTVYGLGF SSEHHLSKFA EKFQEFKEAA RLAKEKSQEK
MELTSTPSQE SAGGDLQSPL TPES

Appendix 3. Homer gene sequence amplified from rat astrocyte mRNA and its corresponding amino acid sequence

ATGGGGGA ACAACCTATC TTCAGCACTC GAGCTCATGT CTTCCAGATT GACCCAAACA
CAAAGAAGAA GTGGGTACCC ACCAGCAAGC ATGCAGTTAC TGTGTCTTAT TTCTATGACA
GCACAAGGAA TATGTATAGG ATAATCAGTC TAGACGGCTC AAAGGCAATA ATAAATAGCA
CCATCACTCC AAACATGACA TTTACTAAAA CATCTCAAAA GTTTGGCCAA TGGGCTGATA
GCCGGGCAAA CACTGTTTAT GGACTGGGAT TCTCCTCTGA GCATCATCTC TCAAAATTTG
CAGAAAAGTT TCAGGAATTT AAAGAAGCTG CTCGGCTGGC AAAGGAGAAG TCGCAGGAGA
AGATGGAACT GACCAGTACC CGTTCACAGG AATCAGCAGG AGGAGATCTT CAGTCTCCTT
TAACACCAGA

MGEQPIFSTR AHVFQIDPNT KKNWVPTSKH AVTVSYFYDS TRNVYRIISL DGSKAIINST

Appendix 4 Homer gene sequence amplified from CHO cells mRNA and its corresponding amino acid sequence

TTCAGCACTC GAGCTCATGT CTTCCAGATT GACCCAAACA CAAAGAAGAA CTGGGTACCC
ACCAGCAAGC ATGCAGTTAC TGTATCTTAT TTTTATGACA GCACAAGAAA TGTATATAGG
ATAATCAGTT TAGATGGCTC AAAGGCAATA ATAAATAGCA CCATCACTCC AAACATGACA
TTTACTAAAA CATCTCAAAA GTTTGGCCAG TGGGCTGATA GCCGGGCAAA TACTGTTTAT
GGATTGGGAT TCTCCTCTGA GCATCATCTT TCCAAATTTG CAGAAAAGTT TCAGGAATTT
AAAGAAGCTG CTCGTCTTGC AAAGGAGAAG TCACAGGAGA AGATGGAACT GACCAGTACA
CCTTCACAGG AATCAGCAGG TGGAGATCTT CAGTCTCCTT TAACACCGAA AGGT

FSTRAHVFQI DPNTKKNWVP TSKHAVTVSY FYDSTRNVYR IISLDGSKAI INSTITPNMT
FTKTSQKFGQ WADSRANTVY GLGFSSEHHL SKFAEKFQEF KEAARLAKEK SQEKMELTST
PSQESAGGDL QSPLTPKG

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What is claimed is:

1. A method for treatment of neuroleptic syndrome or psychosis in a human being comprising administering to said human being a composition comprising an effective amount of a homer expression modifying compound.

2. A method according to claim 1, whereby the psychosis is schizophrenia.

3. A method for treatment of oncological disorders in a human being comprising administering to said human being a composition comprising an effective amount of a homer expression modifying compound.

4. A method for treatment of neuroleptic induced disorders or psychosis in a human being comprising administering to said human being a composition comprising an effective amount of a compound which interacts with metabotropic receptors.

5. A method according to claim 4, whereby the interaction effects an inhibition of the metabotropic receptor.

6. A method according to claim 4, whereby the compound is 2-methyl-6-(2-phenylethenyl)pyridine or 2-methyl-6-(phenylethynyl)pyridine hydrochloride.

7. A method for treatment of neuroleptic maligne syndrome in a human being comprising administering to said human being a composition comprising an effective amount of a compound which interacts with metabotropic receptors and or homer.

8. An isolated nucleic acid as disclosed in appendix 1 to 4.

9. A method of screening of new compounds which modify homer expression using, Hel cells, A-172 cells, U97 cells or glial cells as described in example 3-9.

10. A method of screening of new compounds modifying homer and

11. A method of treatment of CNS disorders in a human being via glial cells comprising administering to said human being a composition comprising an effective amount of a compound, which is able to act on glial cells and which is able to modulate the expression of homer.

12. A method for the treatment of a disease in a human being comprising administering to the said human being a composition comprising an effective amount of a compound inducing homer protein expression or a composition comprising an effective amount of a homer peptide interacting with the homer interaction motif located in the disease-associated-target.

13. A method according to claim 12 where the disease is degenerative disease involving cell degeneration or cell death or apoptosis and the disease-associated-target is human homologue of AFG2 protein.

14. A method according to claim 12 where the disease is neurodegenerative disease including ischemia and stroke and the disease-associated-target is insulin like growth factor binding protein.

15. A method according to claim 12 where the disease is hepatic degenerative processes and the disease-associated-target is interleukin 6 binding protein.

16. A method according to claim 12 where the disease is tissue degenerative processes involving cell death or apoptosis including neurodegenerative disease and ischemia-induced degeneration and the disease-associated-target is cytochrome oxidase or cytochrome P450 XIA1 or topoisomerase I.

17. A method according to claim 12 where the disease is human diseases including brain diseases and tumour progression and the disease-associated-target is GPI-linked NAD-arginine ADP-ribosyltransferase.

18. A method according to claim 12 where the disease is metabolic

19. A method according to claim 12 where the disease is associated to cholesterol production including senile disorders and the disease-associated-target is low density lipoprotein receptor related protein.

20. A method according to claim 12 where the disease is a human neurodegenerative disease and the disease-associated-target is human F-spondin.

21. A method according to claim 12 where the disease is herpes simplex infection and propagation and the disease-associated-target is DNA helicase/primase complex associated protein.

22. A method according to claim 12 where the disease is herpes simplex virus infection and propagation and the disease-associated-target is UL56 protein.

23. A method according to claim 12 where the disease is varicela-zoster virus infection and propagation and the disease-associated-target is serin/threonine-protein kinase.

24. A method according to claim 12 where the disease is sarcoma virus infection and propagation and the disease-associated-target is sarcoma virus receptor.

25. A method according to claim 12 where the disease is japanese encephalitis virus infection and propagation and the disease-associated-target is NS proteins.

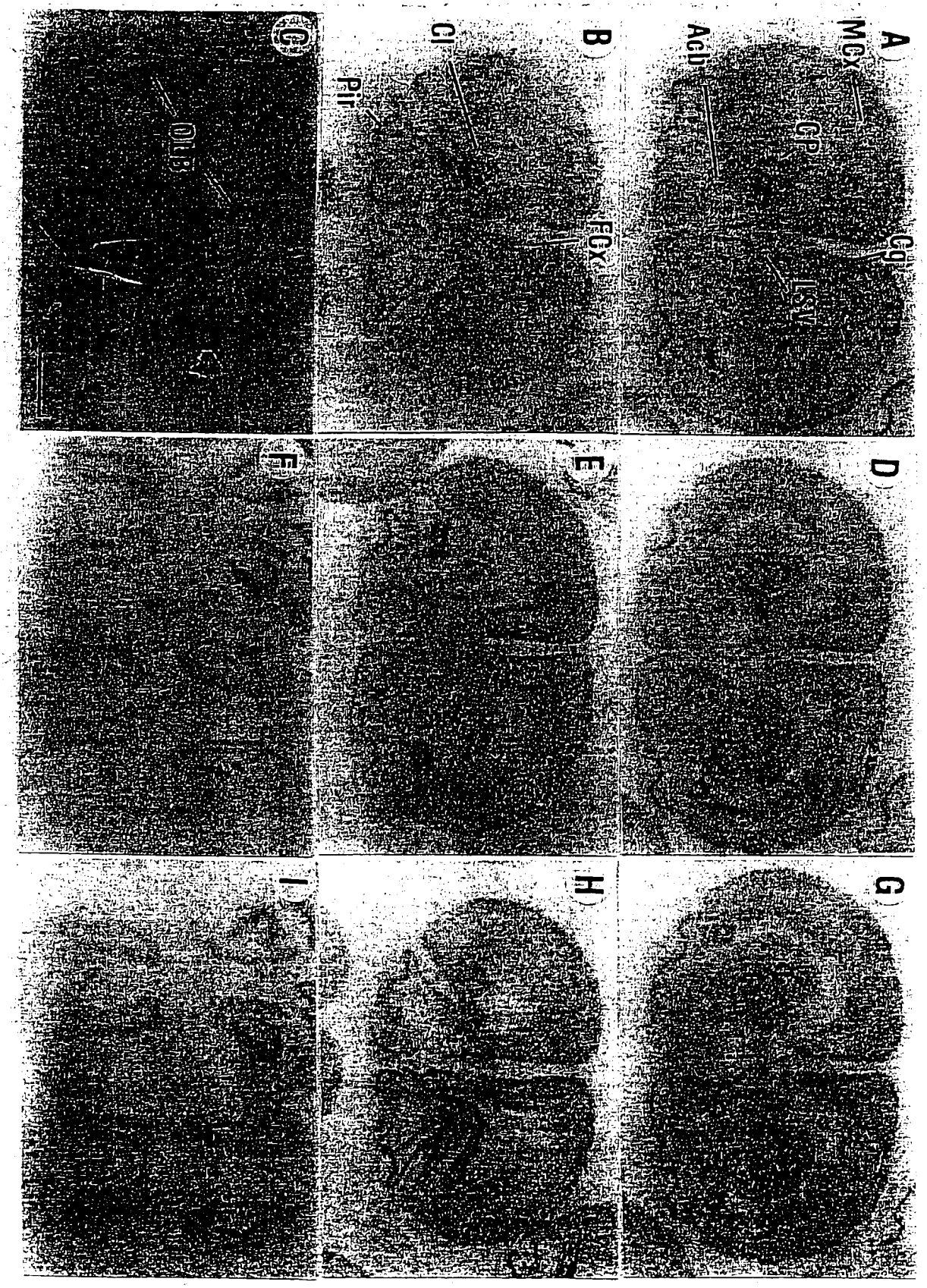
26. A method according to claim 12 where the disease is bovine immunodeficiency virus infection and propagation and the disease-associated-target is virion infectivity factor (factor Q).

27. A method according to claim 12 where the disease is pox virus infection and propagation and the disease-associated-target is protein A11.

28. A method according to claim 12 where the disease is [illegible] and the disease-associated-target is

[illegible] disease-associated-target is topoisomerase I.

Fig. 1



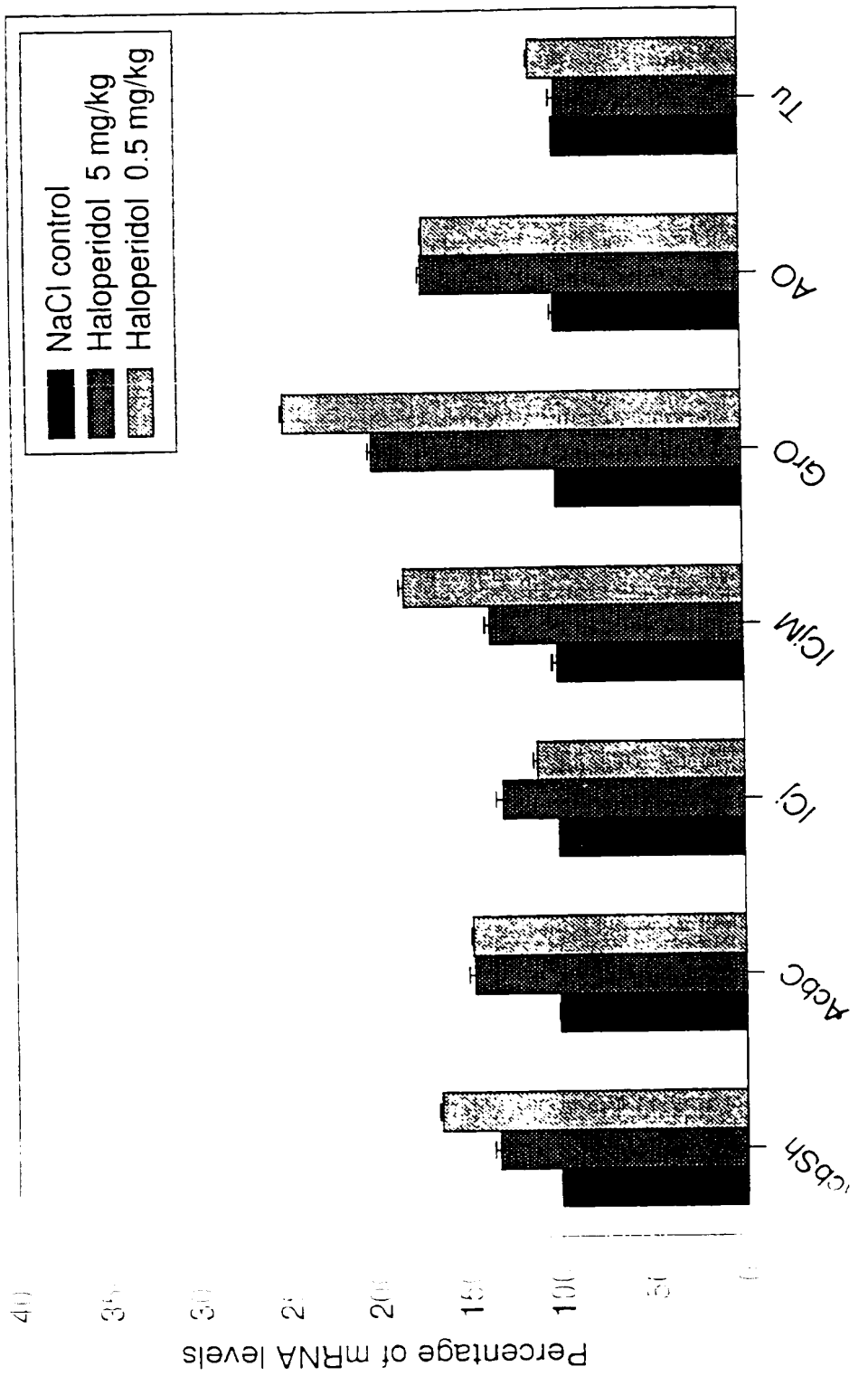

Homer mRNA levels in limbic regions of rat brain
Dose-effect experiment
NaCl control
Haloperidol 5 mg/kg
Haloperidol 0.5 mg/kg
Percentage of mRNA levels
40
30
20
10
0
cbSh
AcbC
ICj
ICjM
Gro
AO
Tu

Fig 1A

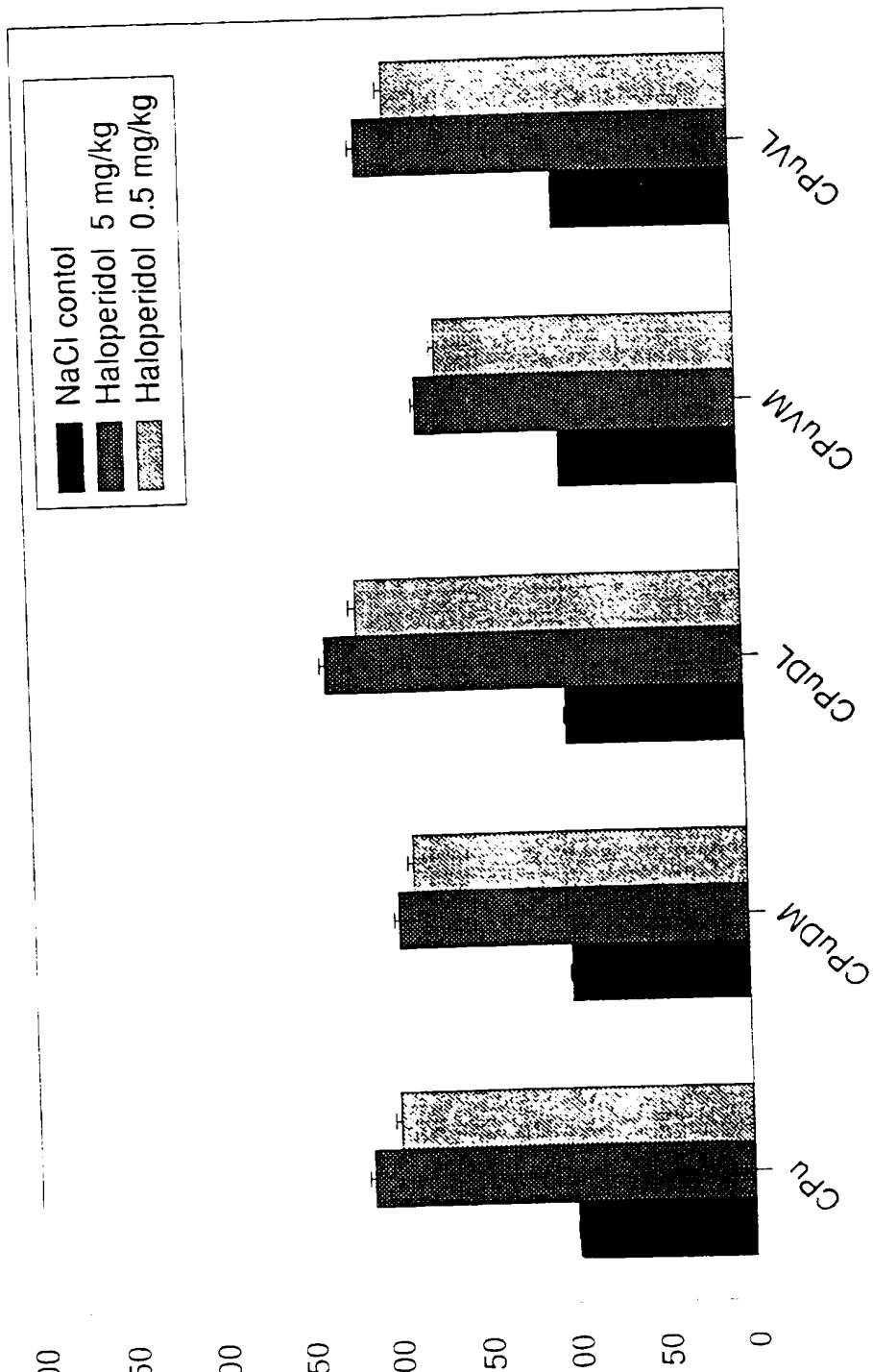

Homer mRNA levels in striatal regions of rat brain
Dose-effect experiment
NaCl contol
Haloperidol 5 mg/kg
Haloperidol 0.5 mg/kg
CPuVL
CPuVM
CPuDL
CPuDM
CPu
400
350
300
250
200
150
100
50
0

Fig 2b

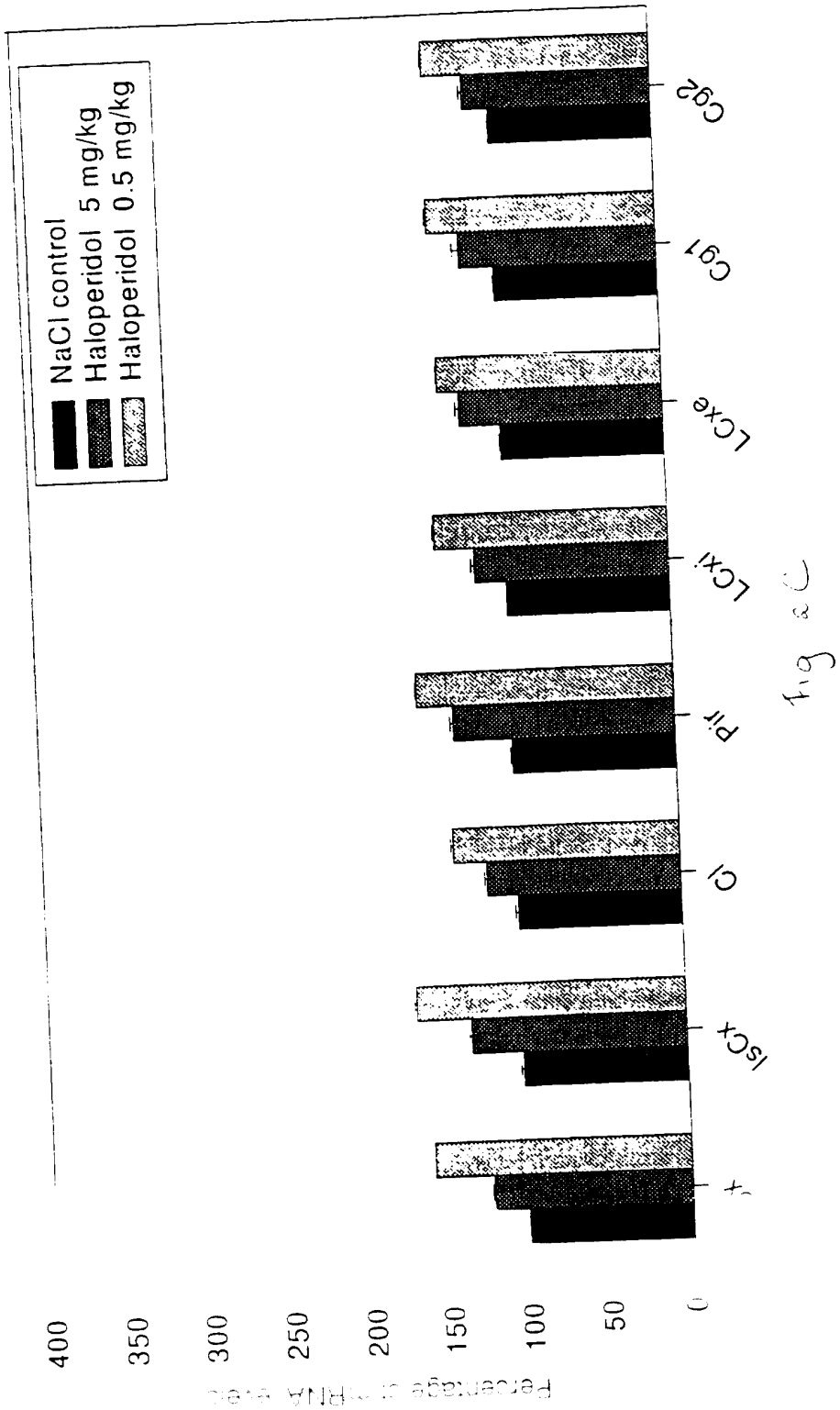

Homer mRNA levels in cortical regions of rat brain
Dose-effect experiment
NaCl control
Haloperidol 5 mg/kg
Haloperidol 0.5 mg/kg
Percentage of mRNA level
400
350
300
250
200
150
100
50
0
Cg2
Cg1
LCxe
LCxi
PIf
Cl
IsCx

fig 2C